Final

December 2002

Bioremediation of oily sludge & soi and ground water pollution monitoring at Mathura Refinery

Prepared for **Indian Oil Corporation Ltd.**
MATHURA REFINERY

TERI Project Report No. 2001MB65

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A suggested format for citing this report is as follows.

TERI 2003
Bioremediation of oily sludge & soil and ground water pollution monitoring at Mathura Refinery
New Delhi. Tata Energy Research Institute. pp.36
[TERI Project Report No. 2001MB65]

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Title of the project	. **Bioremediation of oily sludge & soil and ground water pollution monitoring at Mathura Refinery**
Principal Investigators	: *From TERI, New Delhi* Dr Banwari Lal Dr M R V P Reddy Mr Vinod Kumar *From IOCL, R&D Centre* Dr D K Tuli Dr M P Singh *From Mathura Refinery* Mr A K Saxena
Project duration	: Six months Started on : January 2002 Completed on: August 2002
Project cost	: Rs.10 lakhs
Total oily sludge treated by Oilivorous-S	: 1000 metric tonnes

Contents

1 Executive summary

Indian Oil Corporation Ltd., R & D Centre Faridabad and Tata Energy Research Institute, New Delhi, have signed a MOC on bioremediation of oily sludge.

IOCL, R&D Centre Faridabad and TERI, New Delhi have developed a new bacterial product known as Oilivorous-S which could biodegrade oily sludge at faster rate. Oilivorous –S is developed by blending of five natural occurring bacterial strains which could biodegrade various types of oily sludge. Out of five bacterial strains, two strains biodegrade saturated hydrocarbons fraction of oily sludge. Third strain biodegrades Nitrogen, Sulphur and Oxygen (NSO) containing hydrocarbon fraction of oily sludge, fourth strain biodegrades aromatic and Polycyclic Aromatic Hydrocarbons (PAHs) and fifth strain biodegrades asphaltene fraction of oily sludge. Oily sludge biodegradation efficiency of Oilivorous-S has been tested under laboratory conditions and simulating field conditions. These encouraging results prompted the scientists of IOCL R&D Centre and TERI to use Oilivorous-S at large scale for treatment of oily sludge. Oily sludge treatment by Oilivorous-S is ecofriendly and economical viable approach. The end product of bioremediation of oily sludge by Oilivorous-S is carbon di-oxide and water. Residue after biodegradation of oily sludge contains copious amount of carbonaceous matter (humus) and cell biomass which can be used as soil amender.

Under this work approximately 1000 metric tonne oily sludge was biodegraded by application of Oilivorous-S in six months.

Total 1000 tonnes low oil containing oily sludge was undertaken for biodegradation by using Oilivorous-S. Out of total oily sludge undertaken for biodegradation 93% to 95% oily sludge was biodegraded as compared to target of 90% biodegradation of oily sludge in the proposal.

Similarly 83% to 97% sulphur compounds were biodegraded in oily sludge as compared to target of 70% biodegradation of sulphur compounds in oily sludge.

2 Methods

Production of Oilivorous-S and its application on oily sludge at Mathura Refinery

Indian oil corporation, R & D Centre, Faridabad and Tata Energy Research Institute, New Delhi have developed a new bacterial product known as Oilivorous-S. Oilivorous was developed by assemble of five bacterial species which could biodegrade aliphatic, aromatic, nitrogen, sulphur, oxygen containing compounds and asphaltene fractions of oily sludge. Oilivorous-S (sludge degrading bacterial culture) was produced in a bioreactor at TERI laboratory. Oilivorous-S culture was mixed with a carrier material and carrier based culture was filled in sterilized polybags (packing size 4 kg). Carrier based Oilivorous-S contains 10^{10} cfu/g carrier material and one packet of Oilivorous (4 kg packing size) contains billions of bacteria. Carrier based Oilivorous-S was transported to Mathura Refinery for its application on oily sludge (Figure 1). Oilivorous-S culture (live sludge degrading bacteria) was applied on oily sludge. (Figure 2). Nutrient mixture was also sprayed on oily sludge. After application of Oilivorous-S, mixing of oily sludge and Oilivorous-S bacteria was done by tilling of site with a tractor.

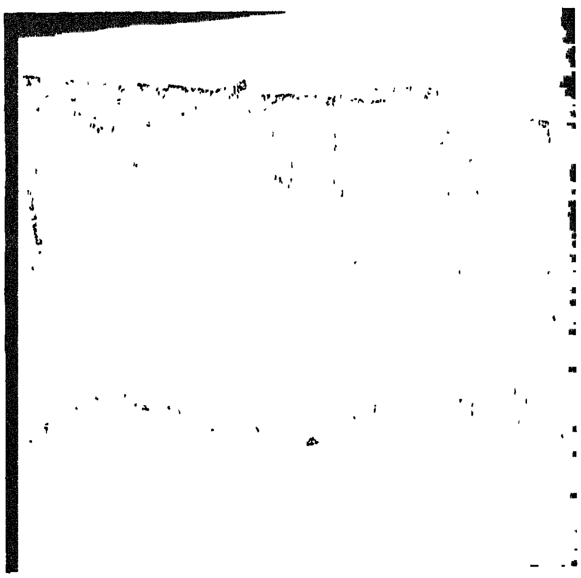


Figure 1. Oilivorous-S (sludge degrading bactenal culture)



Figure 2. Treatment of oily sludge by application of Oilivorous-S at Mathura Refinery

Selection of sites for bioremediation of oily sludge

At Mathura Refinery bioremediation of oily sludge by using a new bacterial product known as Oilivorous-S was initiated in the month of January 2002 and was completed in the month of August 2002. Oilivorous-S can biodegrade oily sludge at very fast rate. At Mathura Refinery two sites (Figure. 3) were selected for bioremediation of oily sludge by using Oilivorous-S. These sites were situated near secure lined sludge pit. The size of site 1 was approximately 5400 m^2 and size of site 2 was approximately 880 m^2. Both the sites were fenced with barbed wire.

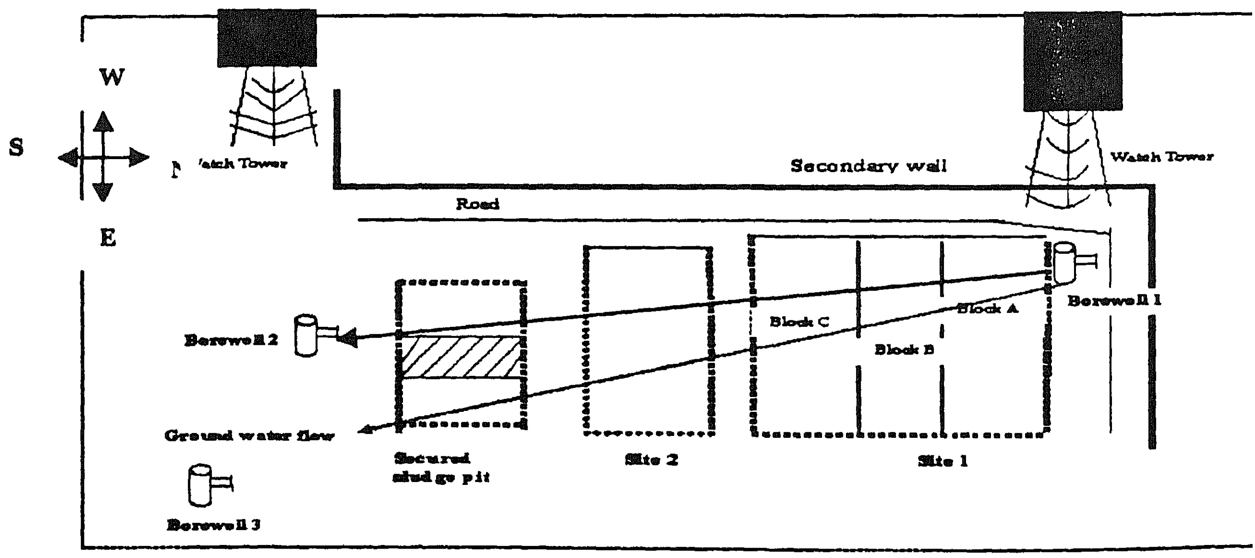


Figure 3. Layout of experimental plots at Mathura Refinery

Characterisation of soil at bioremediation sites

Zero day soil samples were collected from bioremediation site 1 and site 2. Soil samples were collected upto 25 cm depth from both the sites. Soil samples were analysed for water holding capacity, soil bulk density, soil organic carbon, soil total nitrogen, available phosphorus in soil, soil moisture content and soil pH.

Characterization of oily sludge biodegraded at Mathura Refinery

The oily sludge that was biodegraded at bioremediation site 1 & site 2 were analysed. The TPH was extracted from oily sludge using hexane, methylene chloride and chloroform. After solvent evaporation, the oil (TPH) content present in sludge was quantified. The oil (TPH) that was recovered from oily sludge was further fractionated into alkane, aromatic, asphaltanes and NSO (nitrogen, sulphur, oxygen containing compound) by silicagel column. The sediments/sand/silt content in sludge was also estimated after recovery of oil. To estimate the sediment/sand/silt content in residual sludge (after oil recovery), a known quantity of residual sludge (after oil recovery from sludge by solvents) was taken in crucible and heated at 600°C for five hours. After cooling the quantity of sediments/sand/silt recovered from residual sludge was quantified gravimetrically.

Analysis of heavy metals in oily sludge

Selected heavy metals were analysed in oily sludge biodegraded at Mathura Refinery. Oily sludge samples (1 g) collected from bioremediation site was taken in microwave digestion teflon tube. Concentrated nitric acid (15 ml) was added to the Teflon tube and the teflon tube was covered with a watch glass and heated at 140°C on a hot plate, in a fume hood, until most of acid was evaporated, the step was repeated five times in order to solubilise the metallic components. The solution was then filtered in another clean teflon tube through 0.45 μ Whatman filter paper number 42 and the insoluble residues on the filter paper was rinsed with 10% nitric acid. The residue was then discarded. The Teflon tube containing filtered solution was again heated till complete evaporation of acid. After evaporation of acid Teflon tube was heated at 400°C for six hours. Teflon tube was then cooled and the residue was dissolved in 1 ml of concentrated nitric acid. The clear solution was then quantitatively transferred into 50 ml volumetric flask and volume was made by 10% nitric acid. The selected heavy metals from solution was analysed using AAS (Atomic absorption spectrophotometer) as described earlier.

ıd water monıtoring ın upstream and down stream of ʼnedıatıon site

One bore well was installed at upstream ground water flow and two bore wells were installed at down stream ground water flow at bioremediation site. The depths of borewells were 50 feet deep (20 feet from the level of water table). Water from these borewells were flushed out before water sampling. After flushing of water, sample (water samples) were collected in polypropelene bottles. Water samples were collected in duplicates. One set of water samples was kept separately for analysis of pH, oil/grease, conductivity test etc., while other sets of water samples was fixed by HNO_3 (1 N). Both sets of water samples were transported to the IOCL, R&D Centre and TERI, New Delhi for analysis.

Oil/Grease estimation in ground water samples

Oil/grease in water samples were analysed by extraction of total petroleum hydrocarbon (TPH) from water samples using organic solvent. Various fractions of oil/grease were analysed by GC using capillary column. Oil/grease were fractionated into alkane, aromatic, asphaltene and NSO (nitrogen, sulphur and oxygen containing compound) fractions with silicagel column. After dissolving oil/grease in n-pentane, soluble and insoluble fractions (asphaltenes) were obtained. Pentane soluble oil was fractionated into various fractions by silicagel column. A known quantity of pentane soluble TPH was loaded on silicagel column and fractions were eluted with different solvents. The alkane fraction was eluted with hexane, followed by the aromatic fraction that was eluted with benzene. The NSO fraction was eluted with chloroform and methanol. Further, alkane and aromatic fractions were concentrated by evaporation of solvents and then 0.2 µl of each was analyzed by gas chromatography (GC Hewlett Packard, 5890 Series II). All the compounds present in the alkane and aromatic fractions of TPH were identified by GC analysis. In the alkane fraction, various alkane compounds were identified by matching the retention time with authentic standards. Simultaneously, identification of each alkane compound was confirmed by spiking the alkane fraction. A known amount of each standard alkane compound was added to the alkane fraction and injected into GC (co-injection) so that the peak area of each alkane compound increased as a result. Similarly, the polycyclic aromatic hydrocarbon (PAH) compounds were identified by matching the retention time with an authentic standard and confirmed by spiking the sample with authentic standards. Conductivity and pH of water samples were analysed by standard methods.

Analysis of selected heavy metals in ground water collected from Mathura Refinery

As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Se and Zn were analysed in ground samples of borewells situated at upstream and down stream of bioremediation sites. Ground water samples were collected at zero day (just before initiation of bioremediation) and after different time intervals of initiation of bioremediation. Water from borewells were flushed out before collection of samples. Water sample were collected (in duplicate) in polypropeline bottles. One set of water samples in polypropeline bottles were fixed with HNO_3 (1N). Samples were transported to IOCL, R&D Centre and TERI for analysis. Selected heavy metals were analysed in water samples fixed with HNO_3 by atomic absorption spectrometre (AAS).

Digestion

The samples were digested with the help of MARS 5 (Microwave Accelerated Reaction System version 5) Supplied by CEM Corporation, USA. Digestion was completely closed digestion. Five ml of HNO_3 and 1 ml of hydrogen fluoride were used for digestion. The samples were diluted to required volume and then analysis was carried out.

Analysis

Analysis was carried out with the help of AAS- TJA (Unicam, USA) SOLAAR M Series Model fitted with Gilson Flame auto sampler with Auto Diluter and GF95 Graphite Furnace having FS 95 autosampler. Data was processed with SOLAAR M Data station S/W version 8.12, spectrometer S/ W version 1.14.

Wherever needed, matrix modification was performed to overcome the interference of the matrix.

Analysis of Leachable heavy metals in oily sludge

Leachable heavy metals were analysed in oily sludge. A Plastic container (40 litre capacity) was taken. In the bottom of plastic containing a slop of 30° angle was prepared by impermeable material as shown in Figure 4. A tap was fixed in bottom of plastic container to collect Leachate as shown in Figure 4.

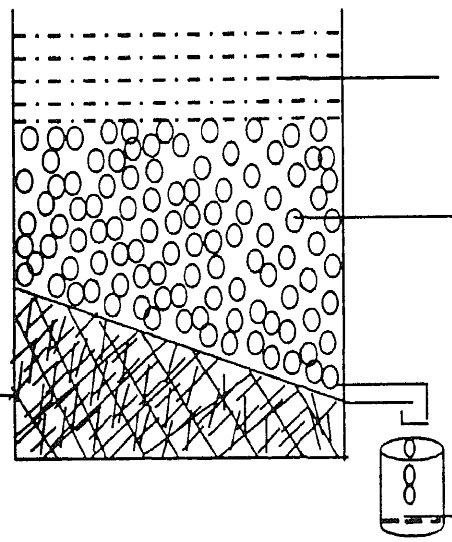


Figure 4. Collection of oily sludge leachate in a plastic container

Oily sludge was filled in this plastic container. Acidified water (with HNO_3, pH 2.0) was poured on oily sludge. The container containing oily sludge and acidified water was kept for 60 days. Leachates were collected in a glass beaker. Selected heavy metals were analysed in Leachates as described above.

Degradation of oily sludge by Oilivorous-S bacteria

At site 1 approximately 750 metric tonne oily sludge was lying while at site 2 approximately 250 metric tonne oily sludge was lying (Figure 5). In the month of January 2002 Oilivorous-S (750 kg) was applied on oily sludge at site 1 and 250 kg Oilivorous-S was applied on oily sludge at site 2. Tilling of Oilivorous-S bacteria and oily sludge was done by a tractor fitted with harrow (Figure 6).

Tilling of oily sludge was also carried out at 30 days intervals for uniform mixing of Oilivorous-S bacteria in oily sludge by using tractor (Figure 6). Tilling also provides air to Oilivorous-S bacteria for faster decomposition of oily sludge.

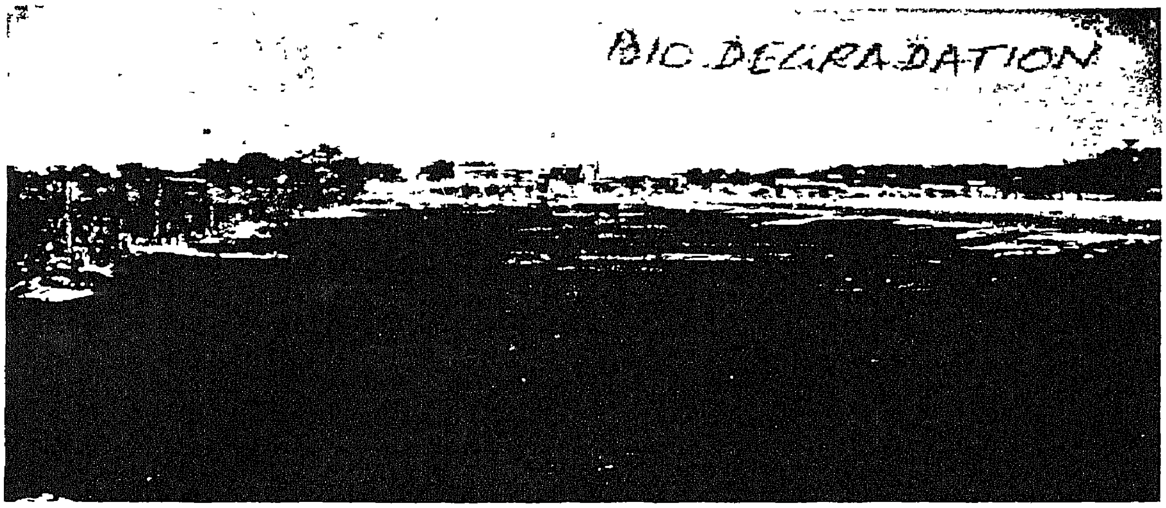


Figure 5. At zero day oily sludge lying at bioremediation site 2

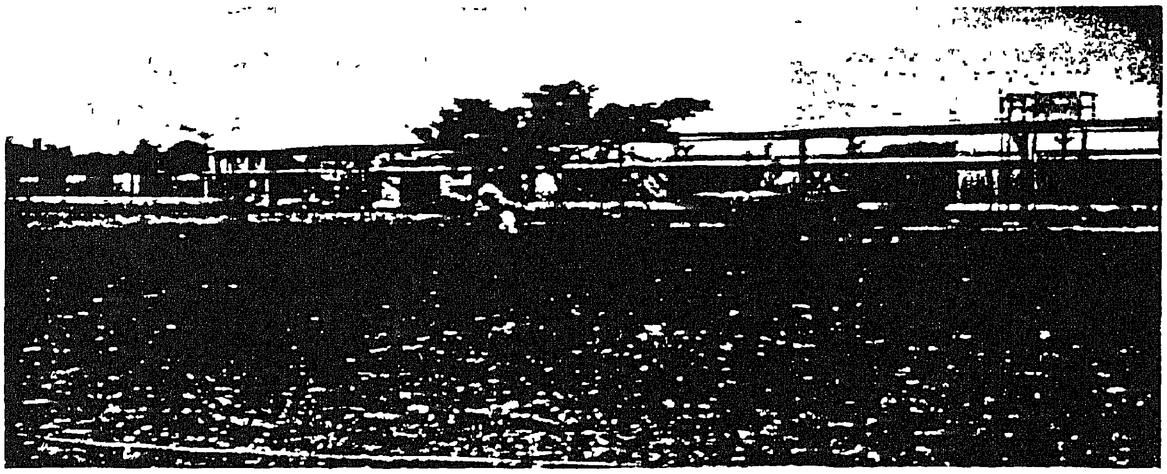


Figure 6. Tilling of site for uniform mixing of Oilivorous-S bacteria on oily sludge

To quantify the rate of degradation of oily sludge by Oilivorous-S, samples of oily sludge was collected from both the sites. On day zero (before treatment o f oily sludge by Oilivorous-S) oily sludge was not uniformally distributed at site 1, therefore, site 1 was divided into three blocks (Block A, B & C). At zero day oily sludge samples were collected from ten points of each block of site 1. At zero day oily sludge samples were also collected from ten points of site 2. Oily sludge samples from Blocks A, B & C of site 1 were collected after different time intervals. Oily sludge sample from site 2 was also collected after different time intervals. Oily sludge samples were taken in polythene bags and transported to IOCL, R&D Centre Faridabad and TERI, New Delhi for analysis.

Total petroleum hydrocarbon (TPH) was extracted from 10 g oily sludge samples collected at zero day and at different time intervals. TPH from 10 g oily sludge samples were extracted with hexane, methylene chloride and chloroform (100 ml each) consecutively. After extraction, all three extract were pooled and dried at room temperature by evaporation of solvents under gentle nitrogen stream in a fume hood. After solvents (hexane, methylene chloride and chloroform) evaporation, the amount of TPH recovered from 10 g oily sludge collected at zero days and after different time intervals was quantified by simple gravimetric method.

Degradation of various fraction of oily sludge

Degradation of various fractions of oily sludge by Oilivorous-S was estimated by fractionation of TPH extracted from oily sludge samples collected at day zero and after different time intervals. The alkane fraction was eluted with hexane, followed by the aromatic fraction that was eluted with toluene. The NSO fraction was eluted with chloroform and methanol. Alkane and aromatic fractions were concentrated by evaporation of solvents and then 0.2 µl of each was analyzed by gas chromatography (GC Hewlett Packard, 5890 Series II). In the alkane fraction, various alkane compounds were identified by matching the retention time with authentic standards. Simultaneously, the identification of each alkane compound was also confirmed by spiking the alkane fraction. A known amount of each standard alkane compound was added to the alkane fraction and injected into GC (co-injection) and the peak area of each alkane compound increased as a result. Similarly, the polycyclic aromatic hydrocarbon compounds were identified by matching the retention time with an authentic standard and confirmed by spiking the sample with authentic standards. Simultaneously, degradation of alkane and aromatic fractions was assessed using a regression factor for each compound, drawn from a standard curve of each compound of alkane and aromatic constructed by GC.

Monitor the TPH contamination in soil at different depths

Contamination of TPH in soil at different depths at bioremediation sites was monitored. Six points (three points from each site) were selected from both the sites for sampling at different depths. Soil samples were collected from 0-25 cm depth, 25-50 cm depth, 50-75 cm depth and 75-100 cm depth by using samplers. These samples were collected in polythene bags and transported to IOCL, R&D Centre Faridabad and TERI, New Delhi for analysis. TPH in these samples collected from different depths were extracted by using solvents as described above.

3 Results

Composition of oily sludge biodegraded at Mathura Refinery

Different types of oily sludge were lying at bioremediation sites. The oil content in the sludge varies from 10% to 56%. Composite samples of oily sludge were analyzed. The composition of composite sample of oily sludge is shown in Table 1. Oily sludge biodegraded at Mathura Refinery showed that steam extractable total petroleum hydrocarbon (TPH) in oily sludge was negligible. However, solvent extractable TPH in oily sludge was 28.32%, water 24%, organic carbon 19.8% and sediments/sand/silt was 27.88%. Oil (TPH) extracted from oily sludge by solvent was fractionated into different fractions showed 56% saturated hydrocarbons fraction, 35% aromatic fraction, 4% NSO fraction and 5% Asphaltene fraction.

Table 1. Composition of oily sludge of Mathura Refinery

Constituents of oily sludge	Composition (%)
Steam extractable total petroleum hydrocarbon in oily sludge	Nil
Solvent extractable TPH in oily sludge	28.32
Water content in oily sludge	24
Organic carbon in oily sludge	19.8
Sediments (sand, silt, etc.)	27.88
Constituents of TPH	
Saturated hydrocarbons fraction	56.0
*Aromatic fraction	35.0
NSO fraction	4.0
Asphaltene fraction	5.0

* Aromatic fraction contains polycyclic aromatic hydrocarbon (PAHs) compounds 12 6% and phenols 0 6% and unsaturated hydrocarbons fractions.

Selected heavy metals in oily sludge biodegraded at Mathura Refinery

Selected heavy metals were analysed in oily sludge biodegraded at Mathura Refinery. As shown in Table 2 the concentration of most of heavy metals in oily sludge was within the permissible limits except Fe. High concentration of iron in

oily sludge may be explained that iron pieces and drums were lying in oily sludge, hence oily sludge was contaminated with iron, therefore, the concentration of iron in oily sludge was more than permissible limits.

Table 2. Selected heavy metals concentration in oily sludge biodegraded at Mathura Refinery

Heavy metals	Concentration of heavy metals in oily sludge (mg/kg oily sludge)
Fe	13.56
Mn	0.44
Zn	2.50
Cu	0.09
Ni	0 03
Pb	0.38
As	0.13
Cd	0 041
Co	0.03
Cr	0.012
Se	0 010

Analysis of heavy metals in leachates of oily sludge

To collect the leachates of oily sludge, a plastic container with a 30° angle slop in the bottom was taken to collect leachate. This container was filled with oily sludge and leachate was collected. In leachate samples heavy metals were analysed. Heavy metals except Fe, Zn, and Ni could not be detected in leachate samples (Table 3). However, concentration of Fe, Zn and Ni in leachate samples was very less.

Table 3. Selected heavy metals concentration in leachates of oily sludge of Mathura Refinery

Heavy metals	Concentration (mg/litre leachate)
Fe	1.96
Mn	BD*
Zn	0.002
Cu	BD*
Ni	0 001
Pb	BD
As	BD
Cd	BD
Co	BD
Cr (Total)	BD
Se	BD

* BD - Below detection limit, Detection limt of AAS is 0.1 ppb

Bioremediation of oily sludge by Oilivorous-S

At site 1 approximately 750 metric tonne oily sludge was lying. In Block A of site 1 at day zero, the oil content in oily sludge was 15.59%. Treatment of oily sludge by Oilivorous-S in Block A resulted that TPH content in oily sludge reduced from 15.59% to 15.10% in one month, 7.59% in 3 months, 3.35% in four months and 0.98% in six months which revealed 93.7% biodegradation of TPH in six months (Figure 7). Biodegradation of TPH in Block A of site 1 was very less in 1st month, however biodegradation of TPH increased significantly in 2nd month (Figure 8). More than half of oily sludge in this block was biodegraded in three months (Figure 8). In four to six months rate of bioremediation of oily sludge by Oilivorous-S was high as compared to first and second month of bioremediation (Figure 8).

In block B of site 1 at day zero the TPH in oily sludge was 13.80%. Treatment of oily sludge by Oilivorous-S in block B resulted that oil content (TPH) in oily sludge reduced from 13.80% to 11.10% in 40 days and 9.80 in two months, 3.40% in four months, 1.90% in five months and 0.96% in six months (Figure 7) which resulted 93% biodegradation of TPH in six months (Figure 8). The rate of biodegradation of oily sludge by Oilivorous-S was slow in first and in second months. In third month, fourth and fifth months rate of bioremediation was almost constant while in six months the rate of bioremediation was the highest (Figure 8).

In block C of site 1 at zero day the TPH level in oily sludge was 20.76% which reduced to 14.05% in more than one month (40 days), 10.20% in two months, 5.70% in three months, 2.80% in four months, 1.20% in five months and 0.91% in six months (Figure 7) which exhibited 95.6% biodegradation of TPH by Oilivorous-S in six months (Figure 8). The rate of bioremediation of oily sludge by Oilivorous-S was comparatively slow in first and second months than the rate of bioremediation in third, four and fifth months (Figure 8). This could be explained that additional nutrient application was done in third, fourth and fifty months, therefore, rate of biodegradation was higher during this period.
At site 2 approximately 250 metric tonnes oily sludge was lying. Treatment of this oily sludge with Oilivorous-S showed that oil content (TPH) in oily sludge reduced from 19.02% to 13.33% in more than one month (40 days), 11.45% in two months, 10.31% in three months, 6.35% in four months, 2.30% in five and 0.99% in six months which resulted 94.7% biodegradation of TPH by Oilivorous in six months (Figure 7).

The rate of bioremediation of oily sludge was slow in first second and third months (Figure 8) The rate of bioremediation was moderate in fourth month. However, rate of bioremediation was high in fifth and six months (Figure 8). The low rate of bioremediation in first, second and third months could be explained that the area of site 2 was very small and sludge loading was more, therefore, tilling of oily sludge could not be carried out as tractor tyres were slipping on oily sludge.

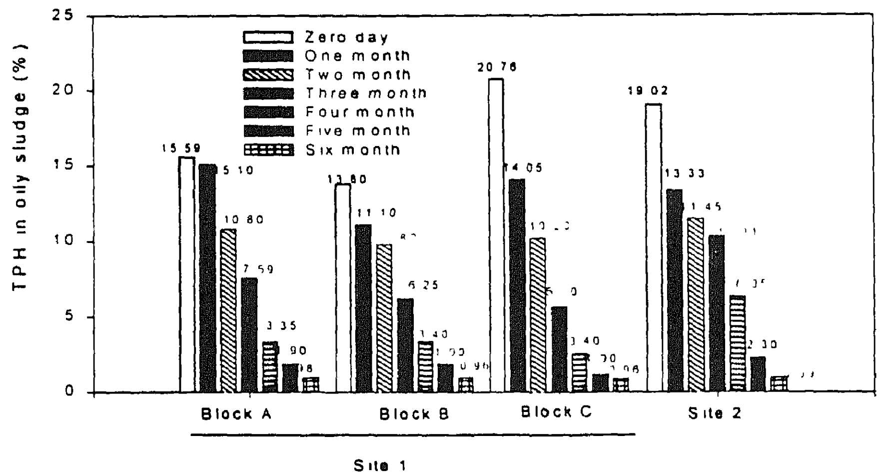


Figure 7. Bioremediation of oily sludge by Oilivorous-S at Mathura Refinery

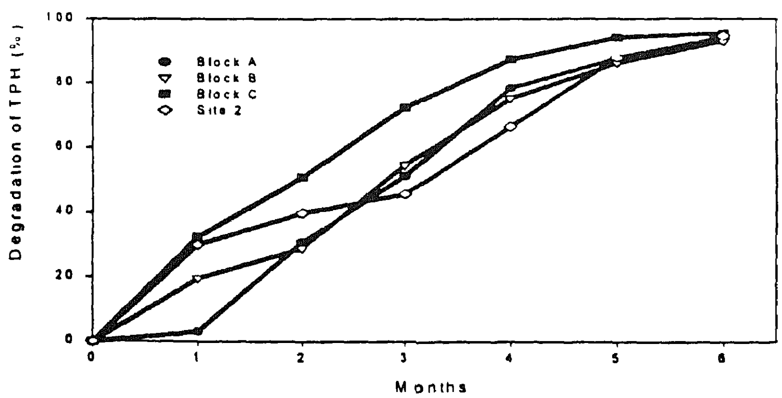


Figure 8. Bioremediation of oily sludge by Oilivorous-S at Mathura Refinery

Biodegradation of various fraction of oily sludge by Oilivorous-S

Oilivorous-S could biodegrade various fraction of oily sludge. In Block A of site 1 Oilivorous-S could biodegrade 93.7% of total petroleum hydrocarbon (TPH) in six months Out of 93.7% degradation of TPH, biodegradation of aliphatic fraction was 54.8% aromatic fraction (including polycyclic aromatic hydrocarbon and phenols) 31.2%, NSO fraction 3.1% and Asphaltene fraction was 4.60% in six months (Table 4). GC finger printing of biodegradation of aliphatic and aromatic fraction of oily sludge at block A is shown in Figure 9 and Figure 10 respectively.

However, in block B of site 1 Oilivorous-S could biodegraded 93% TPH in six months. In block B biodegradation of aliphatic fraction 53.8%, aromatic fraction (PAHs, phenol and aromatic compounds) 31.3%, NSO fraction 3.1% and Asphaltene fraction was 4.8% in six months (Table 4). GC fingerprinting of biodegradation of aliphatic and aromatic fractions in blocks B is shown in figure 11 and figure 12 respectively. Similarly in block C total 95.6% TPH was biodegraded by Oilivorous-S in six months. Biodegradation of aliphatic, aromatic, NSO and Asphaltene fractions in block C were accounted for 54.7%, 32.7%, 3.2% and 5.0% respectively in six months (Table 4). GC finger printing of biodegradation of aliphatic and aromatic fraction of oily sludge at block C is shown in figure 13 and figure 14 respectively.

At site 2 a total 94.7% TPH was biodegraded in six months. At site 2, degradation of aliphatic fraction accounted 53.9%, aromatic fraction accounted 33.8%, NSO fraction was 3.4% and Asphaltene fraction was 3.6% in six months (Table 4). GC finger printing of biodegradation of aliphatic and aromatic fraction of oily sludge at site 2 is shown in figure 15 and figure 16 respectively.

Table 4 Bioremediation of various fraction of oily sludge by Oilivorous-S at site 1 in six months at Mathura Refinery

Sludge Treatment Blocks		Biodegradation (%)				
		TPH	Aliphatic	Aromatic fraction	NSO fraction	Asphaltene fraction
Site 1	A	93.7	54.8	31.2	3.1	4.60
	B	93.0	53.8	31.3	3.1	4 8
	C	95.6	54.7	32.7	3.2	5.0
Site 2		94.7	53.9	33.8	3.4	3.6

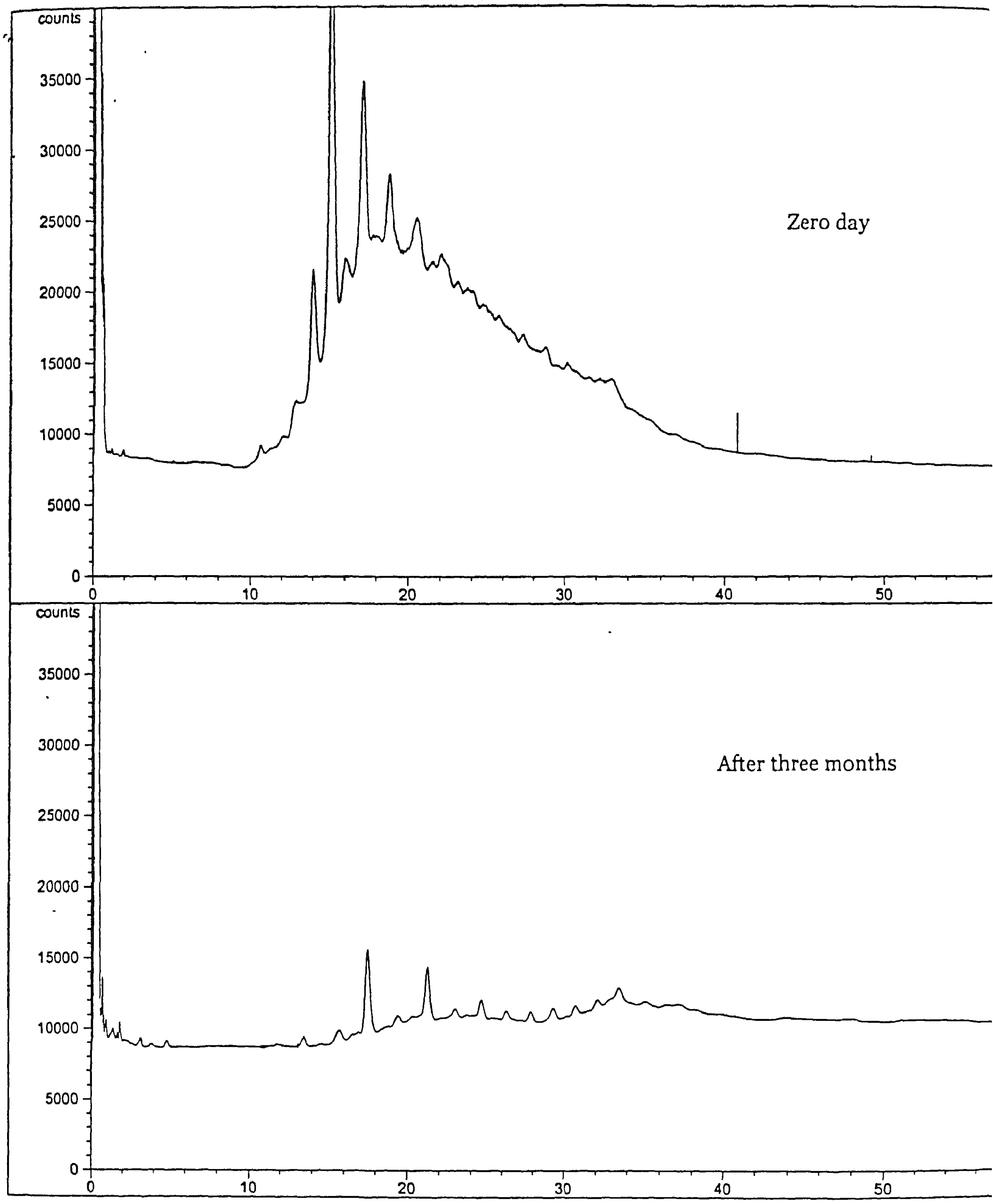


Figure 9. G C Fingerprinting of biodegradation of aliphatic fraction of oily sludge in Block A at Mathura Refinery

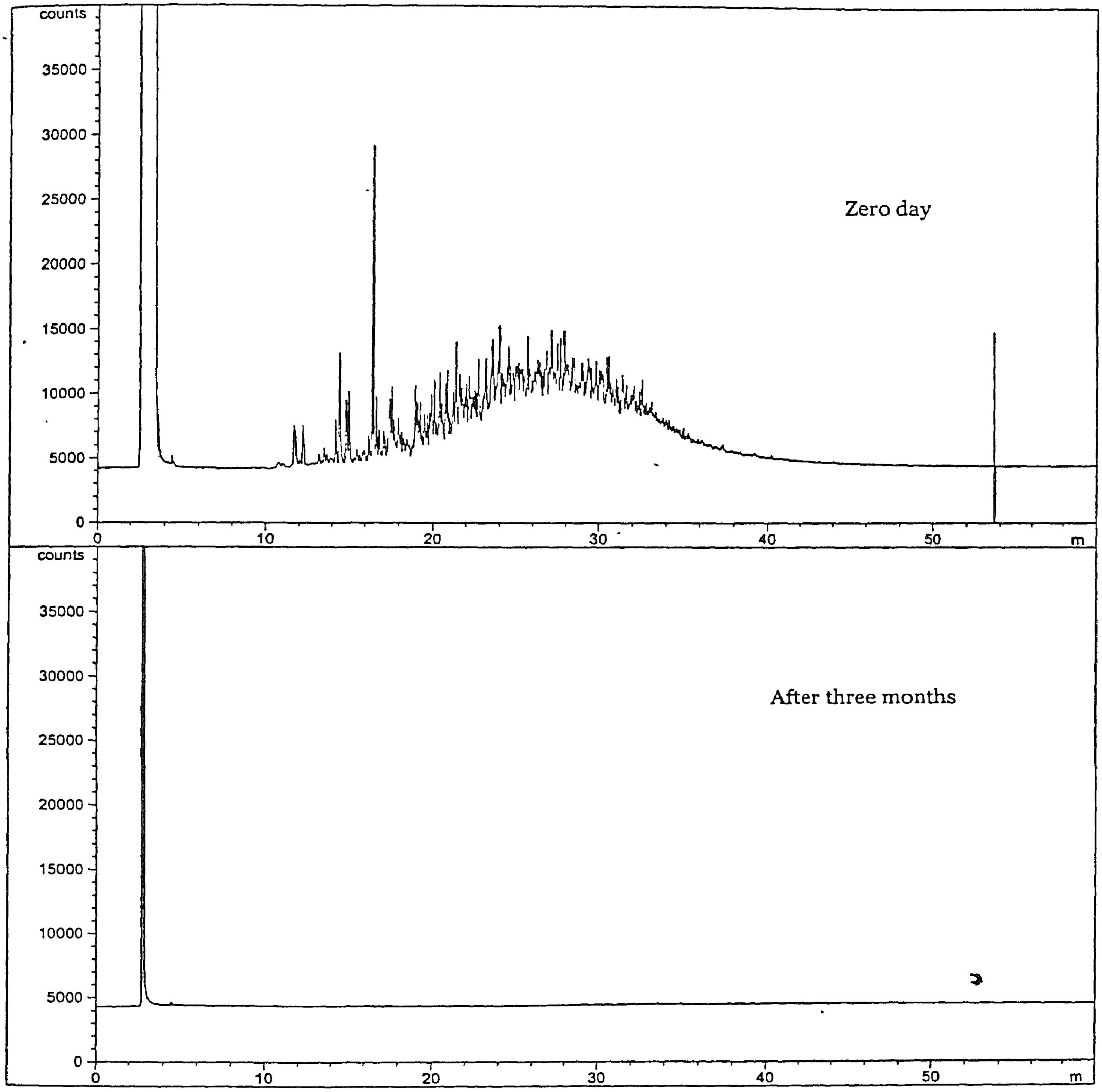


Figure 10. G C Fingerprinting of biodegradation of aromatic fraction of oily sludge in Block A at Mathura Refinery

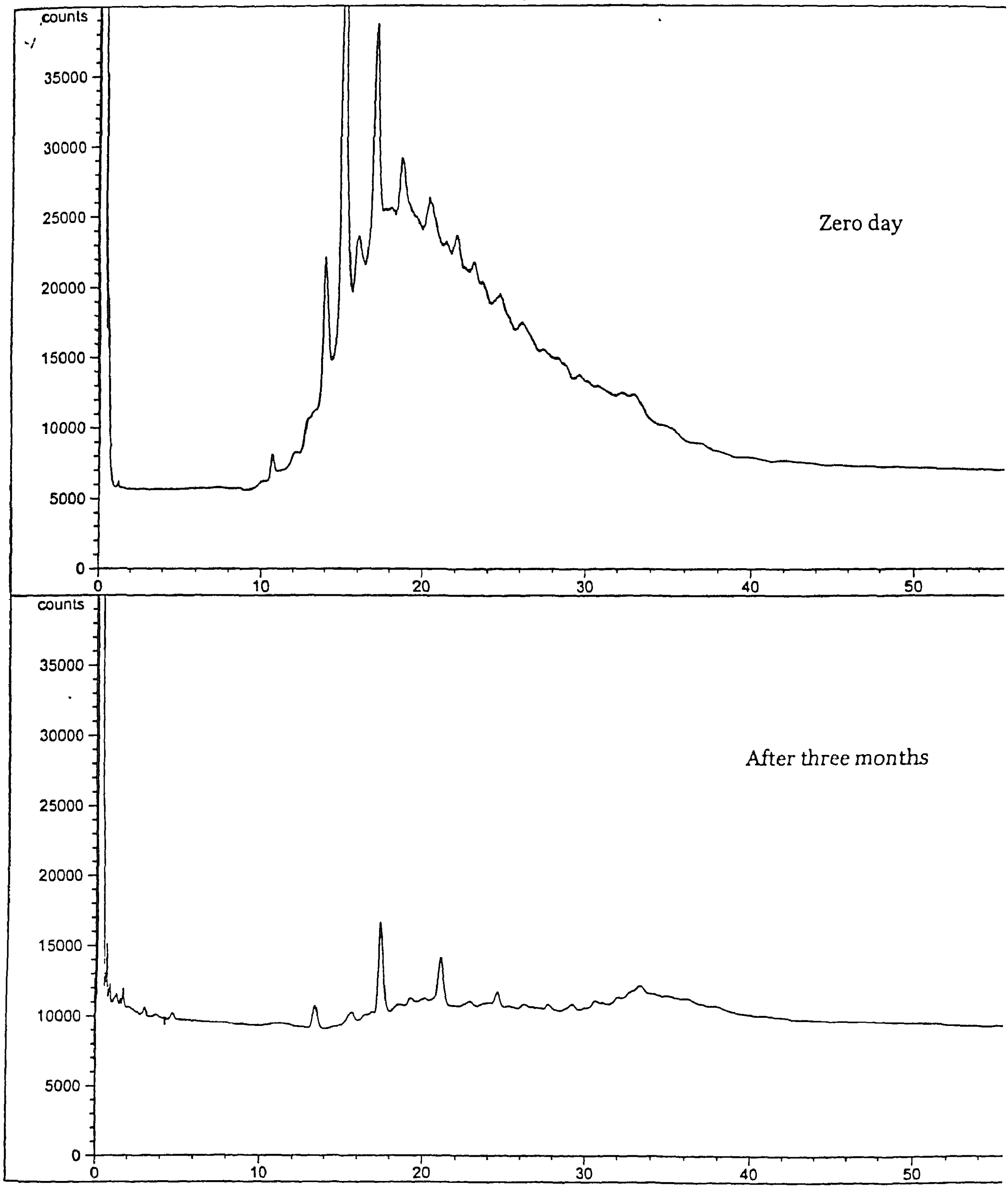


Figure 11. G C Fingerprinting of biodegradation of aliphatic fraction of oily sludge in Block B at Mathura Refinery

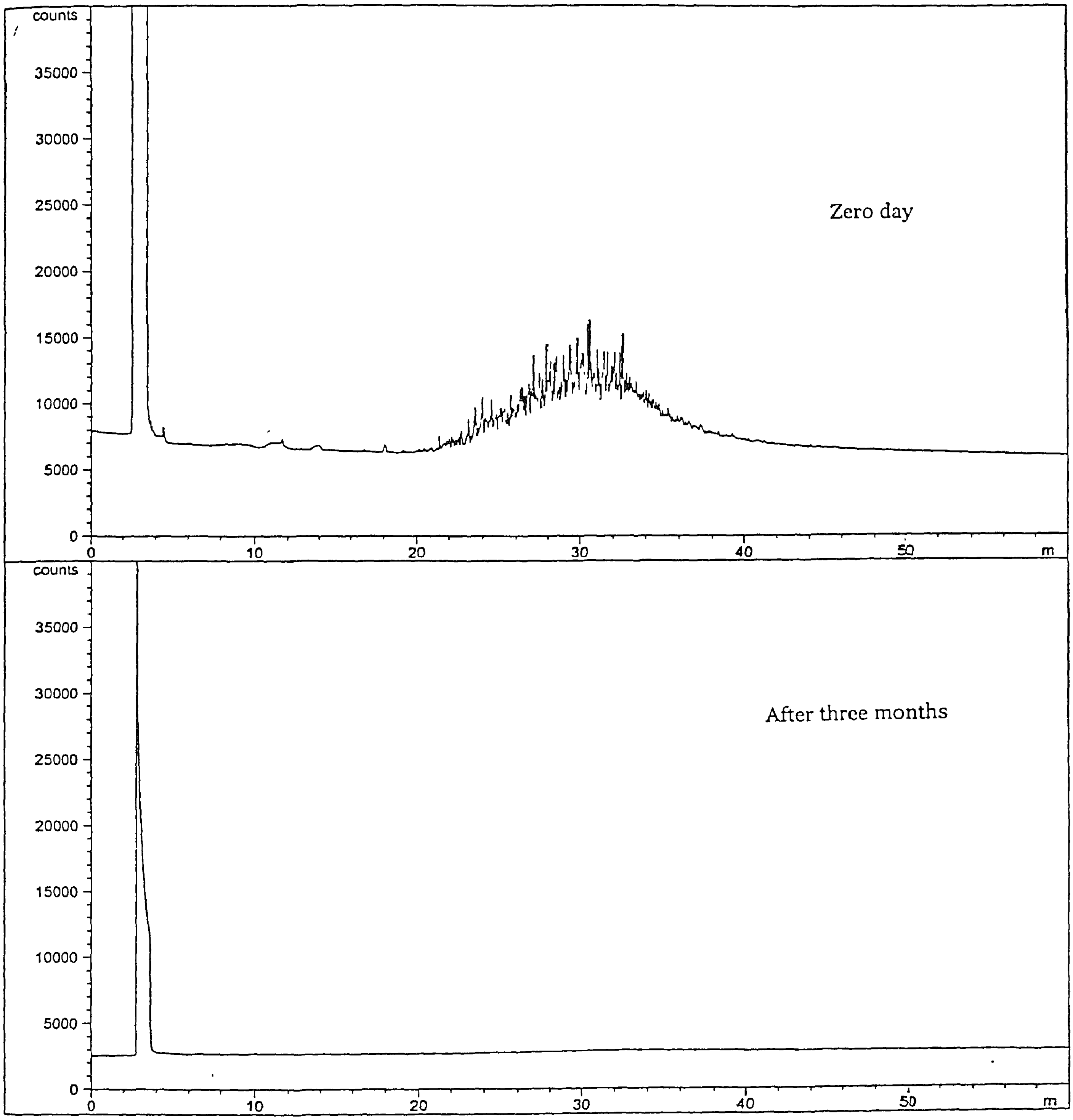


Figure 12. G C Fingerprinting of biodegradation of aromatic fraction of oily sludge in Block B at Mathura Refinery

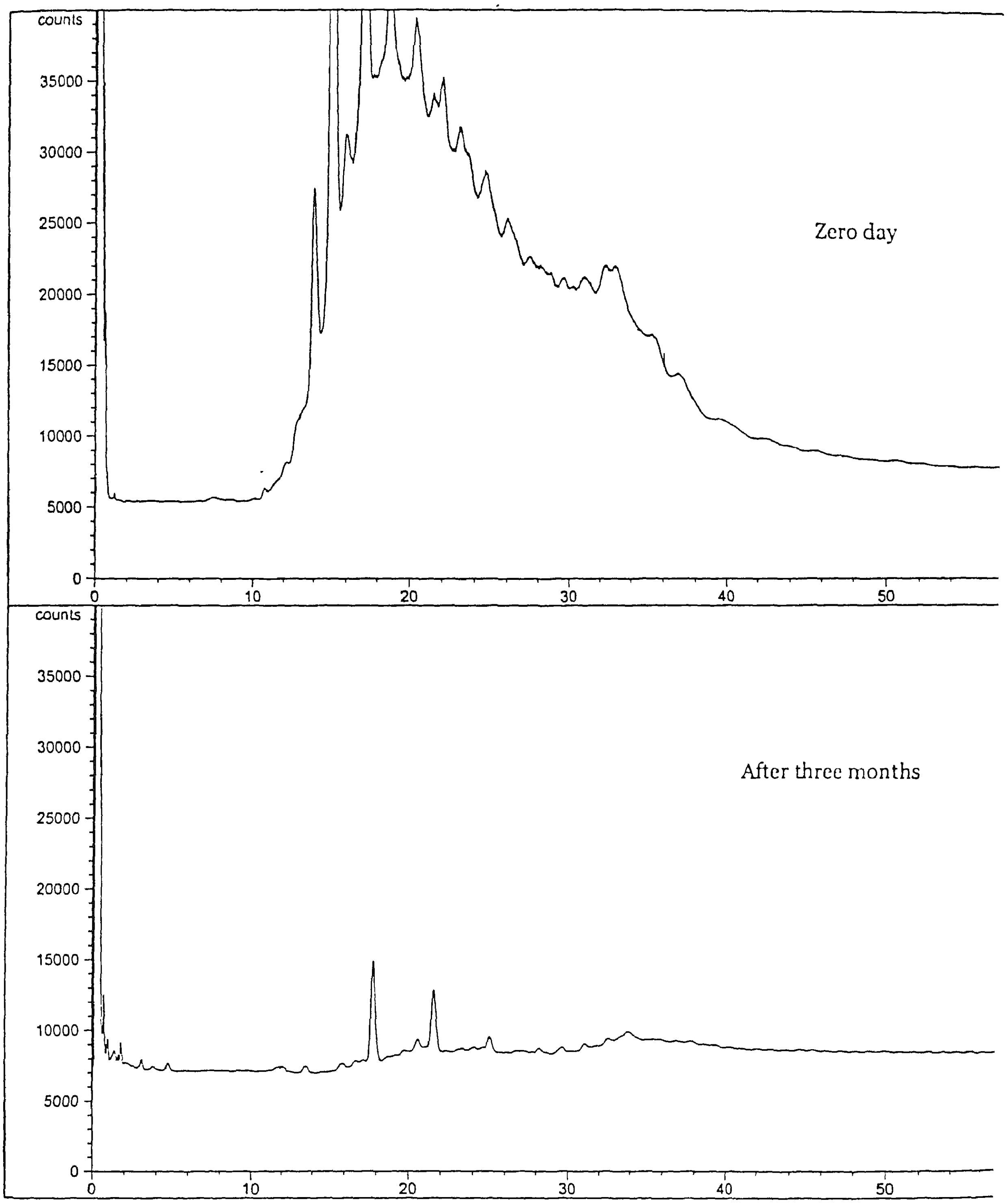


Figure 13 G C Fingerprinting of biodegradation of aliphatic fraction of oily sludge in Block C at Mathura Refinery

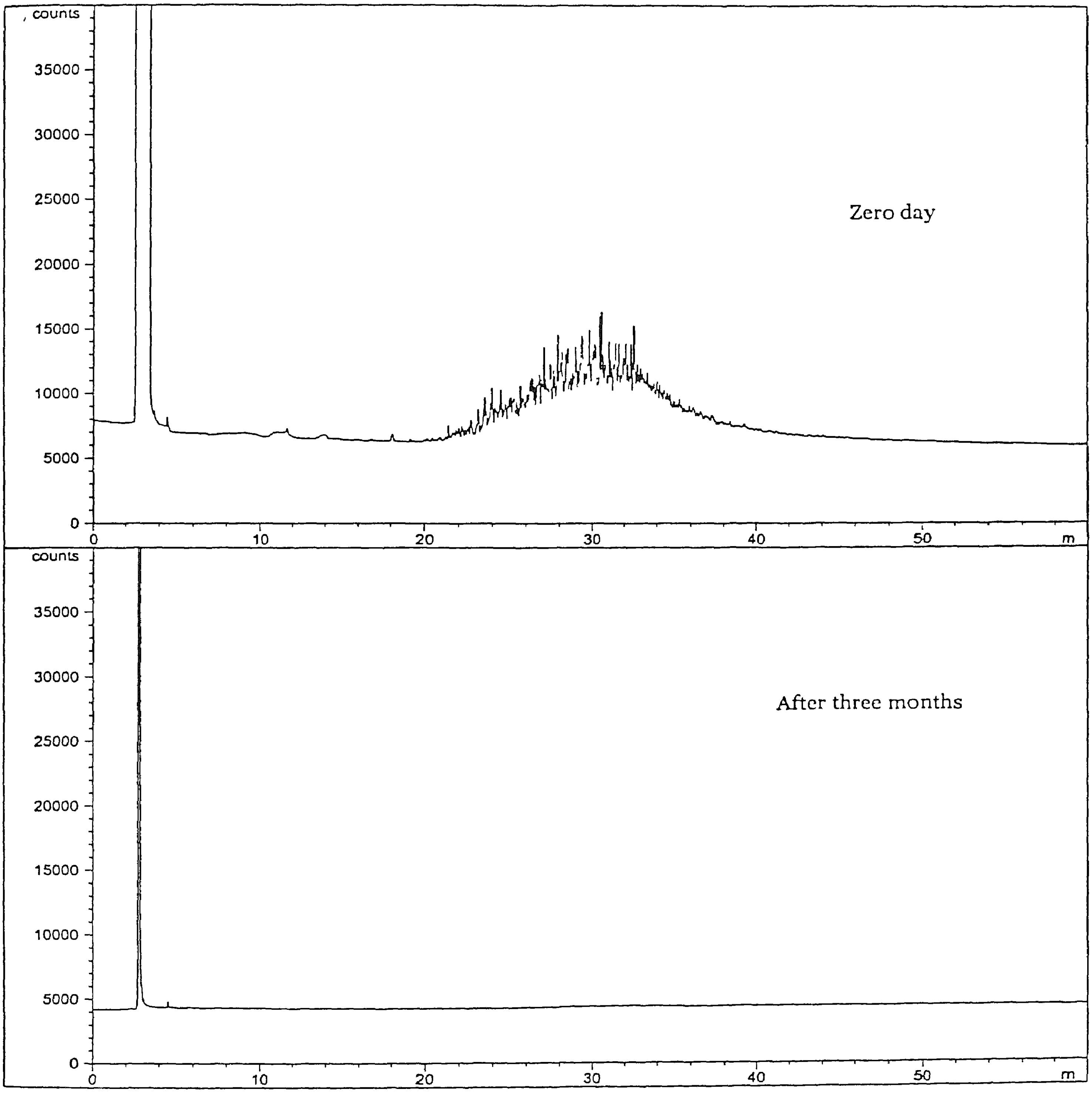


Figure 14. G C Fingerprinting of biodegradation of aromatic fraction of oily sludge in Block C at Mathura Refinery

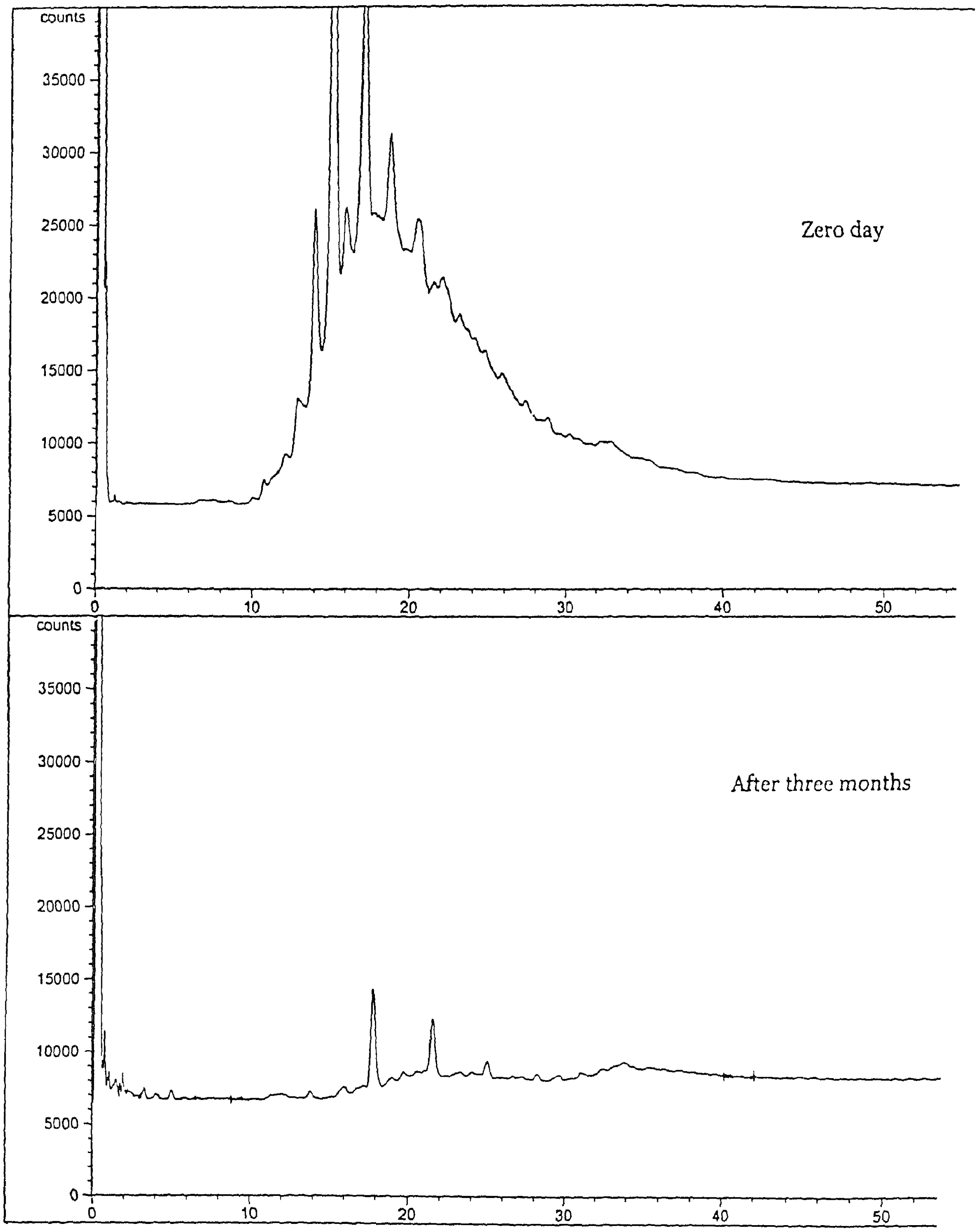


Figure 15. G C Fingerprinting of biodegradation of aliphatic fraction of oily sludge at treatment site 2 at Mathura Refinery

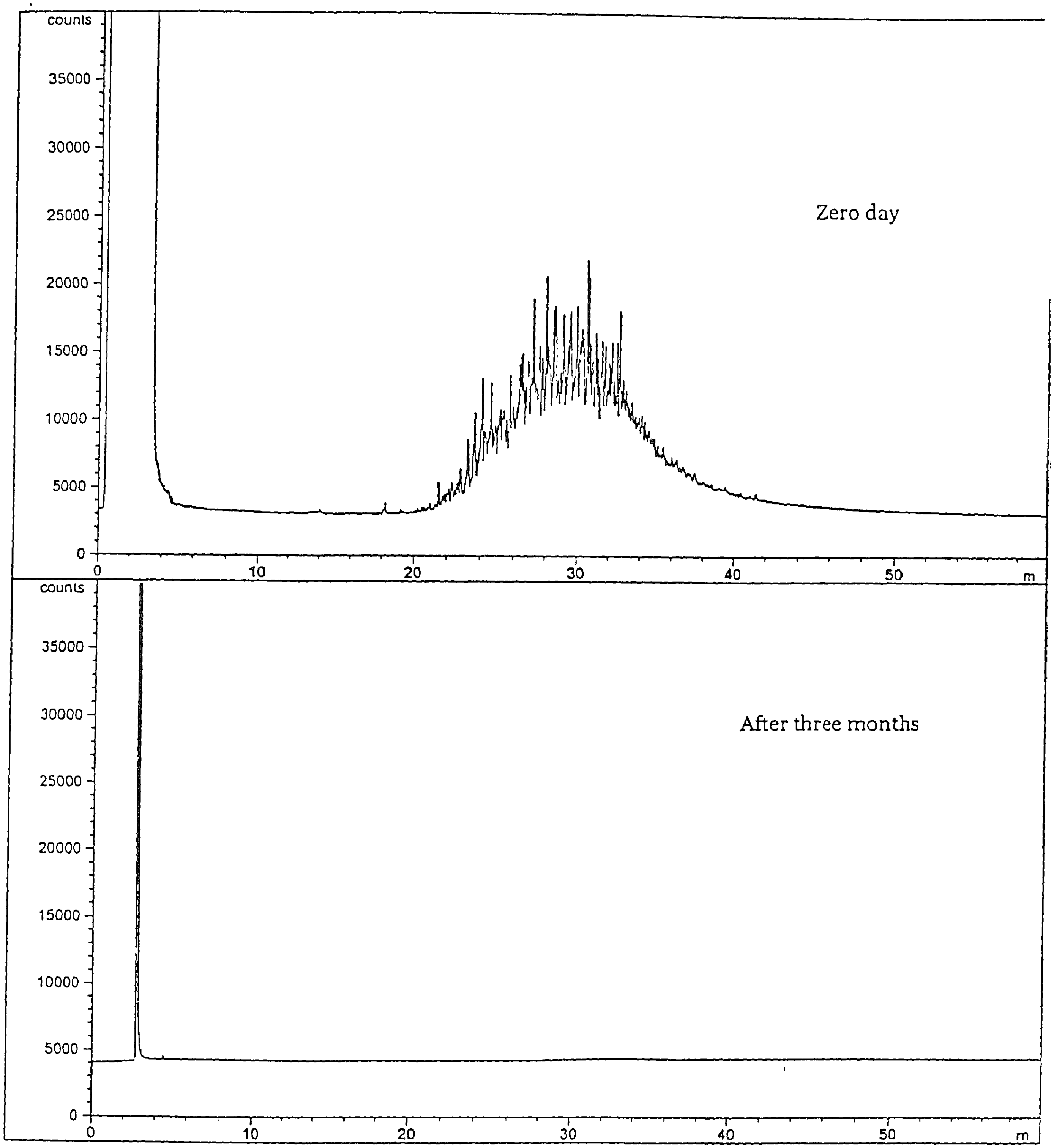


Figure 16. G C Fingerprinting of biodegradation of aromatic fraction of oily sludge at treatment site 2 at Mathura Refinery

Bioremediation sites during different stages of bioremediation at Mathura Refinery are shown in Figure 17, 18, 19 and 20.

Oilivorous-S could biodegrade all the fractions of TPH in oily sludge. After biodegradation of TPH the residue looks like good fertile soil as shown in Figure 21.

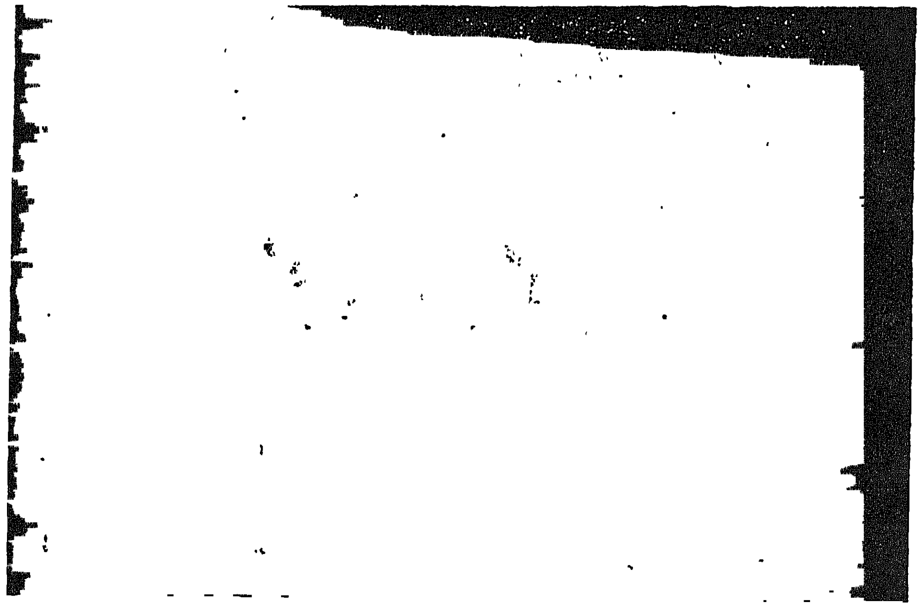


Figure 17. Bioremediation at Mathura Refinery (after one month of initiation of bioremediation)

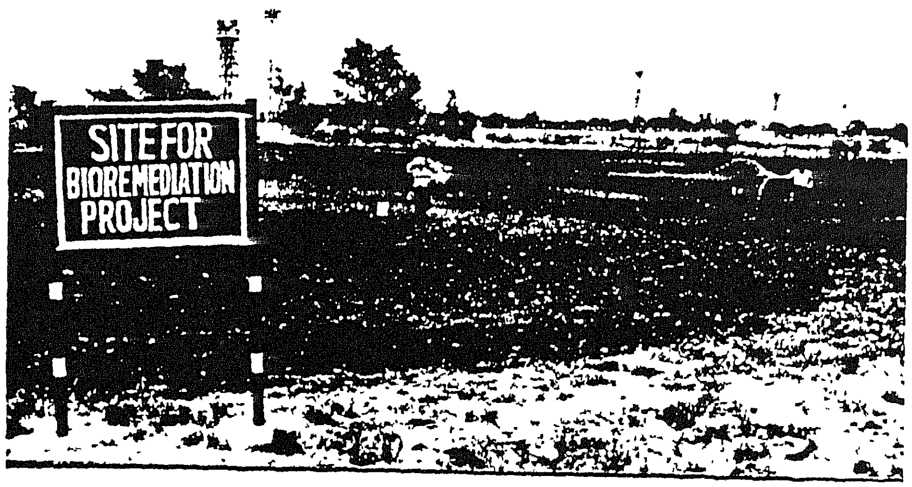


Figure 18. Bioremediation site at Mathura Refinery (after three months)



Figure 19. Bioremediation site at Mathura Refinery (After five months)

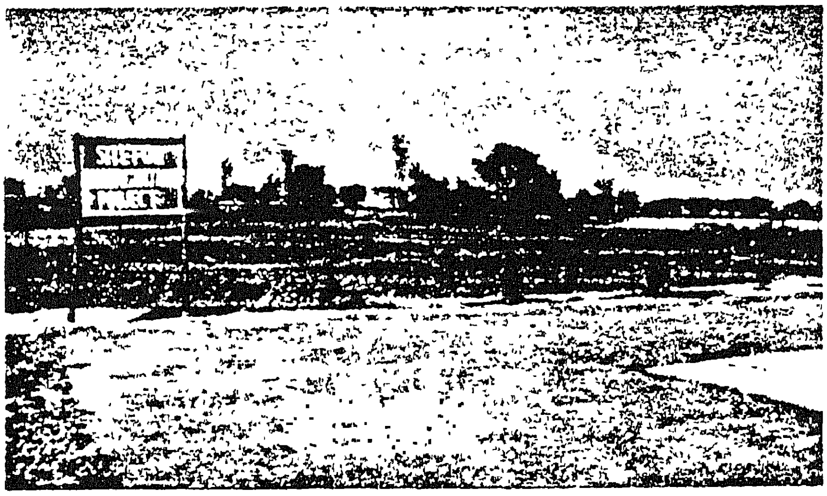


Figure 20. Bioremediation site at Mathura Refinery (after six months).

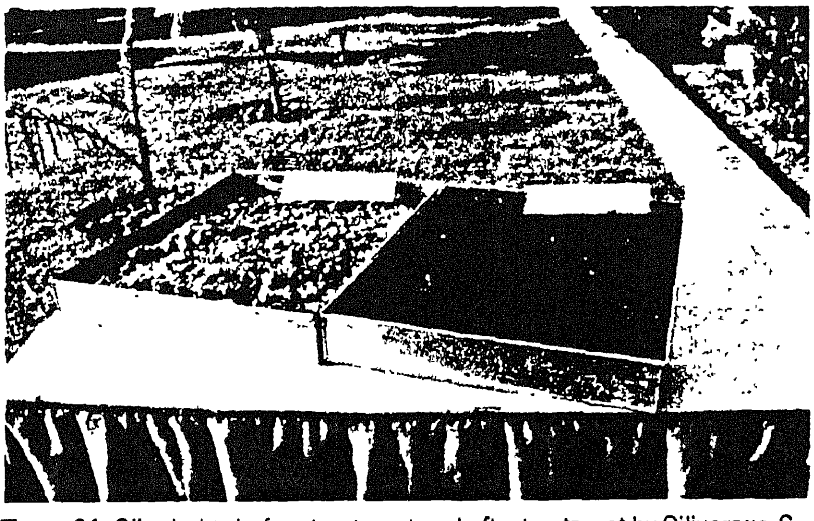


Figure 21. Oily sludge before treatment and after treatment by Oilivorous-S

Biodegradation of sulphur (sulphur compounds) in oily sludge by Oilivorous-S

Bioremediation of sulphurous compounds in oily sludge by Oilivorous-S was monitored. Bioremediation of sulphur compounds by Oilivorous-S is show in Table 5. In Block A 95.8% sulphur compounds were biodegraded by Oilivorous-S in six months. In Block B of site 1 total 83.6% sulphur compounds were biodegraded by Oilivorous-S in same time period. However, in block C, 97.6% sulphur compound were biodegraded by Oilivorous-S in six months (Table 5). Similarly at site 2, 91.2% sulphur compounds were biodegraded by Oilivorous-S in six months (Table 5).

Table 5. Bioremediation of sulphur compounds in oily sludge by Oilivorous-S at Mathura Refinery

Treatment of Plots		Biodegradation of sulphur compounds (%) in six months
Site 1	A	95 8
	B	83.6
	C	97 6
Site 2		91.2

Soil analysis of bioremediation site

At day zero (before initiation of bioremediation) the soil samples were collected from (25 cm depth) site 1 and site 2. At day zero soil analysis of soil site 1 exhibited 2.79% organic carbon, 5.8 ppm available phosphorus, 0.07% total soil nitrogen, 65% water holding capacity of soil, 7.65 soil pH, soil bulk density 1.22 g/cm^3, electric conductivity 0.57 mS/cm and texture was loam (Table 6)

However, soil characteristics at bioremediation site 2 showed that water holding capacity was more than site 1. Organic carbon, total nitrogen and potassium in soil of bioremediation 2 was less than site 1, while electric conductivity and available phosphorous in soil of site 2 was more than site 1 (Table 6).

Soil characteristics after completion of bioremediation at site 1

Soil characteristics at bioremediation site 1 was assessed at zero day and after completion (after six months) of bioremediation work. Soil texture was unchanged during bioremediation of oily sludge (Table 7). There was a slight reduction in soil pH during bioremediation (Table 7). Soil bulk density at bioremediation site 1 was 1.22 g/cm^3 at zero day and 1.20 g/cm^2 and after completion of bioremediation revealed that there was no significant difference in soil bulk density (Table 7). Water holding capacity of soil at bioremediation site 1 was unchanged. Total soil nitrogen at bioremediation site 1 increased significantly during bioremediation (Table 7). Organic carbon, available phosphorous and Potassium in soil at bioremediation site 1 was also increased significantly. The concentration of selected heavy metals in soil at site 1 after completion of bioremediation were almost in the same range as reported at zero days (Table 8). After completion of bioremediation at site 1 the soil characteristics were comparable with good agriculture soil.

Comparison of soil at bioremediation site 2

Soil characteristics at bioremediation site 2 were also monitored during bioremediation. Soil texture and soil pH at bioremediation site 2 were unchanged after completion of bioremediation (Table 9). Soil bulk density decreased slightly (Table 9). Soil water holding capacity was stable after completion of bioremediation at site 2. Total soil nitrogen, organic carbon in soil, potassium and available phosphorous in soil increased significantly showed that the NPK nutrients in soil increased significantly after completion of bioremediation. This could be explained that nutrients were sprayed during bioremediation at both the sites for growth of Oilivorous-S bacteria. Dead cell biomass also acted as nutrients in soil. After completion of bioremediation soil characteristics at both the sites were comparable to the good agriculture soil. Heavy metal concentrations in soil at bioremediation site were unchanged during bioremediation (Table 10).

Table 6 Soil characteristics at Mathura Refinery (At zero time before initiation of bioremediation)

Parameters	Test Results	
	Sampling site 1	Sampling site 2
Texture of soil	Loamy	Loamy
Soil pH	7.65	7 55
Soil bulk density	1.22 g/cm^3	1.21 g/cm^3
Electric conductivity	0.57 mS/cm	0.62 Ms/cm
Soil water holding capacity	65%	71%
Soil permeability (in dry condition)	0.0005 cm/sec	0.0005 cm/sec
Total soil nitrogen	0.07%	0.06%
Organic carbon	2.79%	1.98%
Available phosphorous in soil	5.80 mg/kg soil	6.20 mg/kg soil
Potassium	122 mg/kg soil	117 mg/kg soil

Table 7. Comparison of soil characteristics at bioremediation site 1 at Mathura Refinery

Parameters	Results	
	Zero day	After completion of bioremediation (after six months)
Texture of soil	Loamy	Loamy
Soil pH	7.65	7 35
Soil bulk density	1.22 g/cm^3	1.20 g/cm^3
Soil water holding capacity	65%	67%
Electric conductivity	0 57 mS/cm	0.59 mS/cm
Soil water holding capacity	65%	71%
Soil permeability (in dry condition)	0.005 cm/sec	0 0005 cm/sec
Total soil nitrogen	0.07%	0 08%
Organic carbon	2.79%	3 90%
Available phosphorous in soil	5.80 mg/kg soil	8.4 mg/kg soil
Potassium in soil	122 mg/kg soil	137 mg/kg soil

Table 8. Selected heavy metals in soil at bioremediation site 1 at Mathura Refinery

Heavy metals	Concentration of heavy metal in soil (mg/kg soil)	
	Zero day	After completion of bioremediation
Fe	11.25	11.28
Mn	0.02	0 02
Zn	2.20	2.40
Cu	BD	BD
Ni	0.01	0.01
Pb	0.06	0.05
As	0.05	0.05
Cd	0.02	0.02
Co	0.01	0 01
Cr (Total	0.02	0.01
Se	0.04	0.04

Note BD - Below detection of AAS Detection limit of AAS is 0 1 ppb

Table 9. Comparison of soil characteristics at bioremediation site 2 at Mathura Refinery

Test	Results	
	Zero day	After completion of bioremediation (After six months)
Texture of soil	Loamy	Loamy
Soil pH	7.55	7.42
Soil bulk density	1.21 g/cm^3	1.19 g/cm^3
Electric conductivity	0.62 mS/cm	0.59 mS/cm
Soil water holding capacity	71%	72%
Soil permeability (in dry condition)	0.0004 cm/sec	0.004 cm/sec
Total soil nitrogen	0.06%	0.08%
Organic carbon in soil	1.98%	2.65%
Available phosphorous in soil	6.20 mg/kg soil	9.60 mg/kg soil
Potassium	117 mg/kg soil	138 mg/kg soil

Table 10. Selected heavy metals in soil at bioremediation site 2 at Mathura Refinery

Heavy metals	Concentration of heavy metal in soil (mg/kg soil)	
	Zero day	After completion of bioremediation
Fe	15.42	13.60
Mn	0.03	0.03
Zn	1.32	1.30
Cu	BD	BD
Ni	0.03	0.03
Pb	0.02	0.02
As	0.03	0.024
Cd	0.03	0.03
Co	0.02	0.02
Cr (Total)	0.04	0.04
Se	0.06	0.07

Monitor the contamination of soil at different depth

Soil samples were collected from different depth at bioremediation site as shown in Figure 22. Soil samples were collected from the depth of 25 cm horizon (0-25 cm depth), 50 cm horizon (26-50 cm depth) 75 cm horizon (51-75 cm depth) and 100 cm horizon (76-100 cm depth). At zero day the TPH contamination in 25 cm horizon soil of site 1 was 7.30% which reduced to 0.94% in six months due to degradation of oily sludge by Oilivorous-S (Table 11). Similarly in 50 cm horizon of site 1 the TPH contamination in soil at zero day was 0.14% and 0.05% after six months. However, at 75 cm horizon and 100 cm horizon of site 1 the contamination of TPH in soil could not be detected at zero day and after six months which revealed that oily sludge could not leached in soil. Oily sludge contaminated was restricted to surface soil which was biodegraded by Oilivorous-S.



Figure 22. Collection of soil samples from different depth of bioremediation site at Mathura Refinery

At site 2 the TPH contamination in surface soil (25 cm horizon) was 9.65% at beginning of bioremediation and 0.98% after completion of bioremediation (Table 11). However, at 50 cm horizon (26-50 cm depth) the TPH contamination in soil was 0.16% at zero day and 0.03% after six months (at completion of bioremediation) (Table 11). Below 50 cm horizon in soil the TPH contamination in soil at bioremediation site 2 could not be detected. These findings exhibited that oily sludge could not percolate in soil at bioremediation site 2 (Table 11).

Table 11. TPH contamination (%) in soil at different depth at bioremediation sites at Mathura Refinery

Depth of sampling	Sampling sites			
	Site 1		Site 2	
	Zero day	After six months	Zero day	After six months
25 cm horizon (0-25 cm depth)	7.30	0.94	9.65	0.98
50 cm horizon (26-50 cm depth)	0.14	0.05	0.16	0 03
75 cm horizon (51-75 cm depth)	BD*	BD*	BD*	BD*
100 cm horizon (76-100 cm depth)	BD*	BD*	BD*	BD*

*Below extraction of TPH from soil and analysis of GC. Extraction limit of TPH from soil by soxlcelator 1 ppm.

Ground water monitoring

Ground water monitoring at upstream and downstream of bioremediation site was carried out as shown in Figure 23. Ground water hydrodynamic gradient was parallel to natural water flow on earth surface. Near Mathura Refinery river Yamuna flows from north-west to south-east. Ground water flow at Mathura Refinery was from north-west to south-east side (Data provided by Mathura Refinery, Rites reports). One bore well was installed approximately 20 metre away towards north-west of bioremediation sites. However, two bore wells were installed approximately 70 metre away towards south-east of bioremediation sites (Figure 23). The depth of bore wells was 50 feet (20 feet deep from water table level). Bore well water was flushed out before collection of water samples as shown in Figure 23. Water samples from bore wells were collected in glass bottles. At zero time heavy metal concentration in ground water of test bore well no. 1, 2, and 3 are shown in Table 12. Samples were collected from upstream and down stream test bore wells at zero day and at regular intervals. Oil/grease could not be detected in ground water samples collected at zero day.



Figure 23. Ground water sampling at bioremediation site at Mathura Refinery

After completion of bioremediation the heavy metal concentrations in ground water of upstream and downstream were same (except few heavy metals) as reported at zero time (Table 13, 14 and 15). At zero time concentration of iron in ground water of upstr3eam bore well 1 was 15.37 mg/litre. Iron concentration in ground water of bore well 1 increased from 15.37 mg/litre to 16.50 mg/litre at the time of completion of bioremediation. However, concentration of zinc (Zn) in ground water of borewell 1 situated at upstream of bioremediation site decreased from 1.32 mg/litre to 0.95 mg/litre after completion of bioremediation (Table 13). Concentration of nickel (Ni), lead (Pb), arsenic (As), Cadmium (Cd), Cobalt (CO), and Cromium (Cr) in ground water of borewell no. 1 was remained unchanged after completion of bioremediation (Table 13).

Concentration of heavy metals in ground water of bore well 2 situated at down stream of bioremediation site was remained unchanged after completion of bioremediation, except Iron (Fe), magnesium (Mn) and lead (pb) as shown in Table 14. However concentration of heavy metals in ground water of bore well 3 situated at down stream of bioremediation site remained unchanged after completion of bioremediation except slight increase in concentration of magnise (Mn) and nickle (Ni) as shown in Table 15.

Concentration of zinc (Zn) in ground water of bore well 2 situated at downstream decreased from 1.31 mg/litre to 1.10 mg/litre (Table 14). However, concentration of lead (Pb) in ground water of bore well 2 increased from 0.003 mg/litre to 0.0035 mg/litre and concentration of same in ground water of bore well 3 remained unchanged after completion of bioremediation (Table 15). Concentration of chromium (Cr) in ground water of well no. 2 decreased from 0.002 mg/litre to 0.0015 mg/litre after completion of bioremediation (Table 14). Heavy metals concentration in ground water of well no. 2 and 3 situated at upstream and down stream of bioremediation site were within the permissible limits at zero time and after completion of bioremediation.

pH and conductivity of water samples of borewells 1, 2 and 3 situated at upstream and down stream of bioremediation site were almost in same range at zero day and after completion of bioremediation (Table 13, 14 and 15).

Table 12. At zero day heavy metal concentration in ground water situated at upstream and downstream of bioremediation sites

Heavy metals	Upstream ground water (Well No.1) (mg/litre)	Down stream ground water (Well no.2) (mg/litre)	Downstream ground water (Well no. 3) (mg/litre)
Fe	15.37	15.79	16.10
Mn	0.25	0.23	0.19
Zn	1.32	1.31	1.34
Cu	0.002	0.003	0.003
Ni	0.01	0.01	0.01
Pb	0.003	0.003	0.003
As	0.002	0.003	0.002
Cd	0.001	0.001	0.001
Co	0.001	0.001	0.001
Cr	0.002	0.002	0.002
pH	7.22	7.77	7.78
conductivity	3.35	3.38	3.30

Table 13. Selected heavy metals in ground water of bore well 1 situated at upstream of bioremediation site

Heavy metals	At zero time (heavy metal mg/litre water)	After completion of bioremediation (heavy metal mg/litre water)
Fe	15.37	16.50
Mn	0.25	0.23
Zn	1.32	0.95
Cu	0 002	0.002
Ni	0.001	0.001
Pb	0.003	0.003
As	0.002	0.002
Cd	0.001	0.001
Co	0.001	0.001
Cr	0 002	0.002
pH	7 22	7.13
conductivity	3.35	3.20 mS/cm

Table 14. Selected heavy metals in ground water of borewell no.2 situated at down stream of bioremediation site

Heavy metals	At zero time (heavy metal mg/litre water)	After completion of bioremediation (heavy metal mg/litre water)
Fe	15.79	16.2
Mn	0.23	0.035
Zn	1.31	1.10
Cu	0.003	0.0028
Ni	0.001	0.001
Pb	0.003	0.0035
As	0.003	0.003
Cd	0.001	0.001
Co	0.001	0.001
Cr	0.002	0.0015
pH	7.77	7.48
Conductivity	3.38	3.35 mS/cm

Table 15. Selected heavy metals in ground water of bore well 3 situated at down stream of bioremediation sites

Heavy metals	At zero time (heavy metal mg/litre water)	After completion of bioremediation (heavy metal mg/litre water)
Fe	16.10	15.60
Mn	0.19	0.20
Zn	1.34	1.31
Cu	0.003	0.003
Ni	0.001	0.002
Pb	0.003	0.003
As	0.002	0.002
Cd	0.001	0.001
Co	0.001	0.002
Cr	0 002	0.002
pH	7.78	7.75
conductivity	3.30	3.03 mS/cm

4 Summary

- Treatment of oily sludge by Oilivorous-S resulted that oil content in oily sludge degraded 93%-95% in six months.

- In block A of site 1 biodegradation of total petroleum hydrocarbon (oily sludge) was 93.7% in six months. In Block A the biodegradation of saturated hydrocarbons fraction was 54.8%, aromatic fraction was 31.2%, NSO fraction was 3.1% and asphaltene fraction was 4.60% in six months. In block B of site 1 total 93% total petroleum hydrocarbon of oily sludge was biodegraded in six months. Out of 93% biodegradation of TPH, degradation of saturated hydrocarbons, aromatic, NSO and asphaltene fractions were 53.8%, 31.3%, 3.1% and 4.8% respectively.

- In block C total 95.6% TPH of oily sludge was biodegraded in six months of which biodegradation of saturated hydrocarbons fraction was 54.7%, aromatic fraction was 33.7%, NSO fraction was 3.2% and asphaltene fraction was 5.0%.

- At site 2 total 94.7% TPH was biodegraded in six months. At site 2 biodegradation of saturated hydrocarbons, aromatic, NSO and asphaltene fraction were 53.9%, 33.8%, 3.4% and 3.6% respectively

- Biodegradation of sulphur in oily sludge at Block A, B and C of Site 1 was 95.8%, 83.6%, 97.6% respectively in six months. However, at Site II the sulphur compound in oily sludge was degraded upto 91.2% in three months.

- Oily sludge (hydrocarbon and metal) contamination in soil at bioremediation site 1 and 2 was restricted to surface soil which revealed that oily sludge could not percolate down in soil at bioremediation sites. This is because of good water holding capacity and low permeability of soil. There is considerable improvement in holding capacity of soil after bioremediation.

- Ground water monitoring at bioremediation site revealed that heavy metal concentration in test borewell situated at upstream and downstream of bioremediation site were in the similar range as reported at zero day (before initiation of bioremediation) the permissible limits.

- Soil characteristics at bioremediation site revealed that NPK nutrients in soil at bioremediation increased significantly in six months.